REMARKS/ARGUMENTS

Claims 3-5 and 7-19 were pending in the present application.

Amendment to the Specification

Applicants have amended the specification to add the priority claim.

The action indicates that in order to claim the benefit of a prior-filed application under 35 U.S.C. §§ 365(c) and 119(e), a specific reference to the prior-filed application in compliance with 37 C.F.R. 1.78(a) must be included in the first sentence(s) of the specification following the title or in an application data sheet.

Applicants submit that priority was properly claimed in the initial filing of the PCT application as well as the filing of the subject application under 35 U.S.C. § 371, however, the priority paragraph had not been inserted into the specification. Please refer in **APPENDIX A** for a copy of the front page of the PCT application publication showing that priority indeed was properly claimed. Applicants have amended the specification to include, immediately following the title, the priority data indicated on the filing receipt. Applicants submit that said amendment satisfies §§ 365(c) and 119(e) and respectfully requests that said claim for priority be entered into the record.

Amendment to the Claims

Claim 3 has been amended. Claim 3 has been amended to include subject matter from now cancelled claim 4 and subject matter found in the specification and claims as originally filed, at for example, page 3, line 6 through page 4, line 10; therefore, no new matter has been added. Thus, claims 3, 5 and 7-19 are now pending in the instant application.

New claim 39 has been added to include subject matter found n the specification and claims as originally filed, at for example, page 4, lines 6-11.

Reexamination of the application and reconsideration of the rejections are respectfully requested in view of the above amendments and the following remarks, which follow the order set forth in the Office Action.

Information Disclosure Statement

The action indicates that the information disclosure statements (IDS) submitted on January 11, 2005 and April 28, 2005 have been considered by the Examiner. However, Applicants note that for the IDS submitted on April 28, 2005, sheets 2 of 4 and 3 of 4 have not been initialed, signed or dated by the Examiner. Further, sheet 4 of 4, while signed and dated by the Examiner, does not include the Examiner's initials. Applicants respectfully request, in interest for completeness of the record, that the Examiner initial, sign and date all sheets of the April 28, 2005 IDS.

Claim Objections

According to the action, claims 8-12 are objected to under 37 C.F.R. 1.75(c) as being in improper dependent form for failing to further limit the subject matter of a previous claim. Claim 3 has been amended, *without prejudice*, to include the step of administering the immunosuppressant and the subject matter of cancelled claim 4.

The action further objects to claims 3-5 and 7-19 for various informalities. Applicants have amended, *without prejudice*, claim 3 to insert the word "of" between the terms "protection" and "one."

Applicants submit that in light of these amendments, that the objection of claims 3-5 and 7-19 be withdrawn.

Claim Rejections – 35 U.S.C. § 112

Claims 3-5, 8-12 and 17-19 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. According to the action, the claims do not recite a specific "protective oligodeoxyribonucleotide," but rather refer to an extremely broad genus of oligodeoxyribonucleotides. The action further alleges that the specification does not reasonably provide enablement for a method of administering any protective oligodeoxyribonucleotide to a patient undergoing treatment with any immunosuppressant with the resultant desired effects (see Office Action dated May 23, 2008 at page 6, lines 4-18).

Applicants respectfully traverse this rejection. However, to move prosecution forward, Applicants have amended claim 3, *without prejudice*, to add the step of administering an immunosuppressant, wherein the immunosuppressant comprises a nucleoside and to define the term "protective oligodeoxyribonucleotide."

Specifically, claim 1 as amended now recites in part "...administering to the patent *an immunosuppressant, wherein said immunosuppressant comprises a nucleoside*" and "...a therapeutic effective dose of a protective oligodeoxyribonucleotide,wherein the *protective oligodeoxyribonucleotide is selected from the group consisting of: (a) a polydeoxyribonucleotide corresponding to the following formula of random sequence:* $P_{1\text{-}5}$, $(dAp)_{12\text{-}24}$, $(dGp)_{10\text{-}20}$, $(dTp)_{13\text{-}26}$, $(dCp)_{10\text{-}20}$, *wherein P=phosphoric radical, dAp=deoxyadenylic monomer, dGp=deoxyguanylic monomer, dTp=deoxythymidylic monomer, cDp=deoxycytidylic monomer; or (b) an oligodeoxyribonucleotide having the following physico-chemical and chemical characteristics: molecular weight: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation: +30±48;...*", thereby rendering the rejection moot as it pertains to claim 3.

Further, with regards to the terms "protective oligodeoxyribonucleotide," Applicants argue that the specification is replete with examples and teachings of what the oligodeoxyribonucleotide is protective of. The specification clearly teaches that "administration of a protective oligodeoxyribonucleotide according to the invention is able to protect endothelial cells and epithelial cells from the effects of the immunosuppressant" (see the instant specification at page 6, lines 22-24). The specification further states that "administration of the protective oligodeoxyribonucleotide preferably protects against immunosuppressant-induced side effects, including apoptosis and alloactivation" (*Id.* at page 8, lines 8-9). As can be seen, the specification more than adequately teaches what the term "protective oligodeoxyribonucleotide" encompasses.

With regards to the term "activation," Applicants argue that the specification is replete with examples and teachings of the term "activation" as recited in claim 3. Furthermore, the specification clearly teaches that:

> "The ***activation includes enhanced expression of ICAM-1 and of MHC class I molecules***. The enhancement of expression is preferably substantial. Further preferably, the immunosuppressant induces a ***pro-inflammatory activation of endothelial cells and/or of epithelial cells*** in a patient. The cells are preferably human microvascular endothelial cells (HMEC) and/or dermal and/or alveolar epithelial cells. The damage preferably occurs when the patient's endothelial and/or epithelial cells have been exposed to the immunosuppressant for about 1 hour to about 1 week or more. More preferably, said damage occurs when said cells have been exposed for about 5 hours to about 72 hours. Even more preferably, the duration of such exposure is between 20 hours and 72 hours. Most preferably, the duration of such exposure is more than 48 hours." (emphasis added) (see instant specification at page 6, line 30 through page 7, line 9).

Therefore, it is clear that the specification clearly teaches to one of ordinary skill in the art.

Applicants further argue that the amendment of claim 3 renders moot the rejection that the specification "does not reasonably provide enablement for a method of administering any protective oligodeoxyribonucleotide to a patient undergoing treatment with any immunosuppressant with the resultant desired effects."

In addition, and to speed along prosecution, Applicants direct the Examiner's attention to the enclosed Declaration (Appendix B). In said declaration, Dr. Eissner describes test results that clearly demonstrate that the oligodeoxyribonucleotide according to the invention indeed protects endothelial cells and epithelial cells from immunosuppressant-mediated apoptosis, where the immunosuppressant is a nucleoside. Dr. Eissner, as a co-inventor of the present invention, provides and discusses test results that show that the use of the oligodeoxyribonucleotide of the present invention protects endothelial and epithelial cells from immunosuppressant-mediated apoptosis. Specifically, endothelial cells and tumor cells were cultured in the presence or absence of 5-flurouracil (5-FU) in descending concentrations (ranging from 10 μg/mL to 0.1 μg/mL) in the presence or absence of defibrotide or oligotide for 48 hours (the oligonucleotide had the following physico-chemical and chemical characteristics: MW: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation +30±48). The cells were then harvested and apoptosis measured by propidium iodide staining and FACScan analysis. The results clearly demonstrate the protective effects of the protective oligodeoxyribonucleotide of the present invention. Specifically, endothelial cells treated with 5-FU underwent immunosuppressant-mediated apoptosis while those endothelial cells treated with the oligodeoxyribonucleotide or defibrotide did not. Clearly, the data presented here demonstrate that treatment of HMEC cells with an immunosuppressant claimed in claim 3 induced apoptosis in those cells, however treatment with an oligodeoxyribonucleotide as claimed in claim 3 prevents said apoptosis.

In view of the foregoing amendments and arguments, Applicants respectfully request that the rejection of claims 3-5 and 7-19 under 35 U.S.C. §112, first paragraph be withdrawn.

Claim Rejections – 35 U.S.C. § 102

I. Claims 3 and 7-17 are rejected under 35 U.S.C. §102(b) as being anticipated by Burcoglu et al. (U.S. 5,624,912) (hereinafter Burcoglu). Applicants respectfully traverse this rejection in view of the present

amendment of claim 3, which includes subject matter of now cancelled claims 4, 5 and 7, and for the following reasons.

Claim 3, as amended, recites the administration of an ***immunosuppressant, wherein the immunosuppressant comprises a nucleoside***. Burcoglu teaches treating patients with oligodeoxyribonucleotides, such as defibrotide. No where does Burcoglu teach or reasonably suggest administering an immunosuppressant, much less administering fludarabine.

Anticipation under 35 U.S.C. §102 requires that "each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros., Inc. v. Union Oil Co.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The Burcoglu reference does not meet this standard. Burcoglu fails to describe or suggest all of the elements of claim 3. Claims 8-17 depend from claim 3, and therefore the Burcoglu reference does not anticipate these claims for at least the same reasons as discussed for claim 3. Applicants request that the rejection of claim 3 and 7-17 under §102 in view of Burcoglu be withdrawn.

II. Claims 3, 7-10 and 13-17 are rejected under 35 U.S.C. §102(a) as being anticipated by Sayer et al. (*J. Cancer Res. Clin. Oncol.*, March 2002, 128, pgs 148-12) (hereinafter Sayer). Applicants respectfully traverse said rejection in view of the present amendment of claim 3, which includes subject matter of now cancelled claims 4, 5 and 7, and for the following reasons.

As previously mentioned, claim 3, as amended, recites the administration of an ***immunosuppressant comprising a nucleoside***. Sayer teaches treating patients with methylprednisolone, a corticosteroid, and defibrotide. No where does Sayer teach or reasonably suggest administering an immunosuppressant comprising a nucleoside.

Sayer fails to describe or suggest all of the elements of claim 3. Claims 8-10 and 13-17 depend from claim 3, and therefore the Sayer reference does not anticipate these claims for at least the same reasons as discussed for claim 3. Applicants request that the rejection of claim 3, 7-10 and 13-17 under §102 in view of Sayer be withdrawn.

III. Claims 3-5 and 7-17 are rejected under 35 U.S.C. §102(a) as being anticipated by Bairey et al. (*American Journal of Hematology*, April 2002, 69, pages 281-284) (hereinafter Bairey). Applicants respectfully traverse said rejection in view of the present amendment of claim 3, which includes subject matter of now cancelled claim 4 and for the following reasons.

Bairey teaches treating patients with immunosuppressants such as etoposide (podophyllotoxin) and daunorubicin (antibiotic) after treatment with defibrotide. No where does Bairey teach or reasonably suggest administering an immunosuppressant comprising a nucleoside. As previously mentioned, claim 3, as amended, recites the administration of an ***immunosuppressant comprising a nucleoside***.

Bairey fails to describe or suggest all of the elements of claim 3. Claims 8-17 depend from claim 3, and therefore the Bairey reference does not anticipate these claims for at least the same reasons as discussed for claim 3. Applicants request that the rejection of claim 3-5 and 7-17 under §102 in view of Bairey be withdrawn.

Claim Rejections – 35 U.S.C. § 103

I. Claims 3, 7-16, 18 and 19 are rejected under 35 U.S.C. §103(a) as being unpatentable over Sayer. Applicants traverse the rejection in view of the present amendment of claim 3, which includes subject matter of now cancelled claim 4 and subject matter found in the specification, and for the following reasons.

Claim 3 was amended to recite:

> "A method of treating a patient comprising administering to the patient an immunosuppressant, wherein said immunosuppressant comprises a nucleoside, and a step of administering to the patient a therapeutically effective dose of a protective oligodeoxyribonucleotide, wherein said protective oligodeoxyribonucleotide is selected from the group consisting of:
>
> (a) a polydeoxyribonucleotide corresponding to the following formula of random sequence: $P_{1\text{-}5}$, $(dAp)_{12\text{-}24}$, $(dGp)_{10\text{-}20}$, $(dTp)_{13\text{-}26}$, $(dCp)_{10\text{-}20}$, wherein P=phosphoric radical, dAp=deoxyadenylic monomer, dGp=deoxyguanylic monomer, dTp=deoxythymidylic monomer, cDp=deoxycytidylic monomer; or
>
> (b) an oligodeoxyribonucleotide having the following physico-chemical and chemical characteristics: molecular weight: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455;

> A+G/C+T: 0.800-1.160; specific rotation: +30±48; and achieving protection of one or both of the patient's epithelial or endothelial cells from one or both of apoptosis or activation induced by the administration of the immunosuppressant."

Sayer discloses a method of treating veno-occlusive disease (VOD) by administering high-dose methylprednisolone treatment followed by defibrotide maintenance therapy (see Sayer at Abstract). Hence, the method taught by Sayer is directed to a method for treating VOD which, as clearly explained in the introduction of Bairey, is a non-thrombotic obliteration of small intrahepatic veins by loose connective tissue. No where does Sayer teach or suggest the use of the protective oligodeoxyribonucleotide for protecting epithelial and/or endothelial cells from apoptosis and/or activation induced by the administration of the immunosuppressant as claimed by the Applicants. Moreover, no where does Sayer teach the use of a nucleoside (e.g., fludarabine) as an immunosuppressant. Therefore, Sayer does not teach or reasonably suggest a method of treatment comprising an immunosuppressant, wherein the immunosuppressant comprises a nucleoside, and protective oligodeoxyribonucleotide is selected from the group consisting of: (a) a polydeoxyribonucleotide corresponding to the following formula of random sequence: $P_{1\text{-}5}$, $(dAp)_{12\text{-}24}$, $(dGp)_{10\text{-}20}$, $(dTp)_{13\text{-}26}$, $(dCp)_{10\text{-}20}$, wherein P=phosphoric radical, dAp=deoxyadenylic monomer, dGp=deoxyguanylic monomer, dTp=deoxythymidylic monomer, cDp=deoxycytidylic monomer; or (b) an oligodeoxyribonucleotide having the following physico-chemical and chemical characteristics: molecular weight: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation: +30±48; for achieving protection of epithelial and/or endothelial cells from apoptosis and/or activation induced by the administration of the immunosuppressant.
Applicants note that KSR did not completely reject the teaching, suggestion and motivation standard.[1] The court specifically held that in formulating a rejection using 35 U.S.C. §103(a) based upon a combination of prior art elements, it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed.

Furthermore, Applicants vigorously argue that the results obtained with the parameters as defined in

[1] See *e.g.*, *Ortho-McNeil Pharmaceutical Inc. v. Mylan Laboratories*. 86 USPQ2d 1196 (Fed. Cir. 2008) (ct. held that the TSM test, flexibly applied, merely assures that the obviousness test proceeds on the basis of evidence – teachings, suggestions (a tellingly broad term), or motivations (an equally broad term) – that arise before the time of invention as the statute requires. As KSR requires, those teachings, suggestions or motivations need not be always written references by may be found within the knowledge and creativity of ordinary skilled artisans).

claim 3 yield unexpected results,[2] and therefore could not have been predicted with a reasonable expectation of success, for the following reasons:

(1) Defibrotide protects HMEC cells from F-ara-induced apoptosis. Human dermal microvascular endothelial cells CDC/EU.-HMEC-1 (HMEC) either untreated or treated with an immunosuppressant (e.g., 2-Fluoroadenine 9-beta-D-arabinofuranoside (F-ara) in the presence or absence of varying concentrations of defibrotide (100 μg/mL to 0.1 μg/ml) and assessed for programmed cell death after 48 hours using flow cytometric analysis showed that defibrotide alone as a second control did not influence endothelial cell viability, while defibrotide in the presence of F-ara resulted in a does-dependent protection of F-ara-induced cell death (see instant specification at Example 2, Figures 1A, 2A and 2B). Moreover, pretreatment of HMEC cells for 1 hour with defibrotide resulted in protection of HMEC cells from F-ara-induced apoptosis (see instant specification at Figure 2C);

(2) Defibrotide does not interfere with the anti-leukemic and anti-PBMC effect of F-ara. Primary peripheral blood derived acute myeloid leukaemic (AML) cells with a blast amount of 70% treated with F-ara and cultured in the presence or absence of defibrotide showed that F-ara-mediated cell death was approximately 80%, demonstrating that defibrotide, in contrast to the protective effect on endothelial and epithelial cells, was unable to protect the AML cells (see instant specification at page 16, lines 1-12 and Figure 4A). Similar results were obtained with peripheral blood mononuclear cells (PBMCs) – treatment of PBMCs with F-ara followed by defibrotide resulted in 40.1% undergoing apoptosis as compared to those PBMC cells left untreated, again demonstrating that unlike with endothelial and epithelial cells, defibrotide was unable to protect these cells from F-ara-induced apoptosis (see instant specification at page 16, lines 14-21 and Figures 4B); and

(3) F-ara upregulates intercellular adhesion molecule 1 (ICAM-1) on HMEC with antagonistic effects by defibrotide. HMEC cells cultured in the presence of F-ara for 24, followed by flow cytometric analysis showed an increase in the expression of ICAM-1 on the surface of the HMEC cells (see instant specification at page 16, lines 25-30; Figures 5A and 5B). In contrast, flow cytometric analysis of HMEC cells cultured in the presence of F-ara in the presence or absence of descending concentrations of defibrotide showed that defibrotide in fact antagonized the F-ara-induced ICAM-1 expression of the HMEC cells (see instant specification at page 16, line 30-page 17, line 5; Figure 5C).

[2] See, *e.g.*, *Ortho-McNeil Pharmaceutical Inc.* (demonstrating that unexpected results are objective indicia that provide independent evidence of obviousness).

Therefore, Applicants clearly demonstrate with conclusive data that the elements and method in claim 3 show advantageous properties with respect to the prior art.

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 3, 7-16, 18 and 19.

II. Claims 3-5 and 7-17 are rejected under 35 U.S.C. §103(a) as being unpatentable over Bairey in view of De Luca et al. (*Int. J. Cancer*, 73, pgs. 277-282) (hereinafter De Luca). Applicants traverse the rejection in view of the present amendment of claim 3, which includes subject matter of now cancelled claims 4, 5 and 7, and for the following reasons.

As previously described, claim 3 was amended to recite:

> "A method of treating a patient comprising administering to the patient an immunosuppressant, wherein said immunosuppressant comprises a nucleoside, and a step of administering to the patient a therapeutically effective dose of a protective oligodeoxyribonucleotide, wherein said protective oligodeoxyribonucleotide is selected from the group consisting of:
>
> (a) a polydeoxyribonucleotide corresponding to the following formula of random sequence: $P_{1\text{-}5}$, $(dAp)_{12\text{-}24}$, $(dGp)_{10\text{-}20}$, $(dTp)_{13\text{-}26}$, $(dCp)_{10\text{-}20}$, wherein P=phosphoric radical, dAp=deoxyadenylic monomer, dGp=deoxyguanylic monomer, dTp=deoxythymidylic monomer, cDp=deoxycytidylic monomer; or
>
> (b) an oligodeoxyribonucleotide having the following physico-chemical and chemical characteristics: molecular weight: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation: +30±48; and achieving protection of one or both of the patient's epithelial or endothelial cells from one or both of apoptosis or activation induced by the administration of the immunosuppressant."

Bairey also discloses a method of treating veno-occlusive disease (VOD) by administering defibrotide (see Bairey at Abstract). Again, as clearly explained in the introduction of Bairey, VOD is a non-thrombotic obliteration of small intrahepatic veins by loose connective tissue. Again, there is no teaching or suggesting the use of the protective oligodeoxyribonucleotide for protecting epithelial and/or endothelial cells from apoptosis and/or activation induced by the administration of the immunosuppressant as claimed by the Applicants. Moreover, no where does Bairey teach the use of a

nucleoside (e.g., fludarabine) as an immunosuppressant.

De Luca is asserted in the action to remedy the deficit of Bairey with regard to the immunosuppressant. The action cites De Luca for the teaching of 5-FU by stating that it would have been obvious to practice the method of Bairey by administering defibrotide when the patient is undergoing treatment with 5-FU (see Office Action dated May 23, 2008 at page 22, lines 6-7). However, De Luca fails to remedy the deficiency of Bairey, namely, that Bairey does not teach or suggest the use of the protective oligodeoxyribonucleotide and immunosuppressant that comprises a nucleoside for protecting epithelial and/or endothelial cells from apoptosis and/or activation induced by the administration of the immunosuppressant as claimed by the Applicants.

Therefore, Bairey, alone or in combination with De Luca, does not teach or reasonably suggest a method of treatment comprising an immunosuppressant, wherein the immunosuppressant comprises a nucleoside, and protective oligodeoxyribonucleotide is selected from the group consisting of: (a) a polydeoxyribonucleotide corresponding to the following formula of random sequence: $P_{1\text{-}5}$, $(dAp)_{12\text{-}24}$, $(dGp)_{10\text{-}20}$, $(dTp)_{13\text{-}26}$, $(dCp)_{10\text{-}20}$, wherein P=phosphoric radical, dAp=deoxyadenylic monomer, dGp=deoxyguanylic monomer, dTp=deoxythymidylic monomer, cDp=deoxycytidylic monomer; or (b) an oligodeoxyribonucleotide having the following physico-chemical and chemical characteristics: molecular weight: 4000-10,000 Da; hyperchromicity parameter: <10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation: +30±48; for achieving protection of epithelial and/or endothelial cells from apoptosis and/or activation induced by the administration of the immunosuppressant.

Moreover, and as detailed above, applicants teach that the method as claimed in claim 3 demonstrates unexpected results. Specifically, (1) defibrotide protects HMEC cells from F-ara-induced apoptosis; (2) defibrotide does not interfere with the anti-leukemic and anti-PBMC effect of F-ara; and (3) F-ara upregulates intercellular adhesion molecule 1 (ICAM-1) on HMEC with antagonistic effects by defibrotide.

Therefore, Applicants clearly demonstrate with conclusive data that the elements and method in claim 3 show advantageous properties with respect to the prior art.

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 3, 7-16, 18 and 19 under 35 U.S.C. §103(a).

Petition for Extension of Time/Fees Payable

Applicants hereby petition for a three (3) month extension of time, extending the deadline for responding to the May 23, 2008 Office Action from August 23, 2008 to November 23, 2008. The fee of $555.00 specified in 37 C.F.R. §1.17(a)(3) for said three (3) month extension is hereby enclosed.

The total fee of $555.00 is being paid by Electric Funds Transfer. Authorization is hereby given to charge any deficiency in applicable fees, or credit any overcharges, for this response to Deposit Account No. 13-4365 in the name of Moore & Van Allen, PLLC.

CONCLUSION

Applicants believe that claims 3-5 and 7-19 are in form and condition for allowance. If any additional issues remain, the Examiner is requested to contact the undersigned attorney at (919) 286-8000 to discuss same.

Respectfully submitted,

Guenther Eissner et al.
(Applicants)

Date: November 21, 2008

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APPENDIX A

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/101468 A1

(54) Title: METHOD FOR THE PROTECTION OF ENDOTHELIAL AND EPITHELIAL CELLS DURING CHEMOTHERAPY

(57) Abstract: The present invention is directed to the use of a protective oligodeoxyribonucleotide for the treatment of a patient undergoing treatment with an immunosupressant. The invention is further directed to a pharmaceutical composition containing a therapeutically effective dose of an immunosuppressant and of a protective oligodeoxyribonucleotide.

APPENDIX B

Attorney Docket No.: 021149.000001

Appln. No.	:	10/516,381
Applicants	:	Guenther Eissner, *et al.*
Filed	:	June 10, 2005
Art Unit	:	1635
Conf. No.	:	4749
Examiner	:	Amy Hudson Bowman
Docket No.	:	021149.000001
Customer No.	:	24239
Title	:	Method for Protection of Endothelial and Epithelial Cells During Chemotherapy

Declaration Under 37 C.F.R. §1.132

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

GUENTHER EISSNER declares that:

1. He is a co-inventor of and is familiar with the present U.S. patent application Serial No. 10/516,381, filed June 10, 2005 in the name of Guenther Eissner and Ernst Holler and entitled "Method for Protection of Endothelial and Epithelial Cells During Chemotherapy" and is familiar with the Official Action dated May 23, 2008 issued therein and with the prior art references cited in the Official Action, including the Burcoglu *et al.* (U.S. Patent No. 5,624,912), Sayer *et al.* (*J. Cancer Res. Clin. Oncol.*, March 2002, 128, pgs. 148-152), Bairey *et al.* (*American Journal of Hematology*, April 2002, 69, pgs. 281-284), and De Luca *et al.* (*Int. J. Cancer*, 1997, 73, pgs 277-282) references.

2. He received a Bachelor of Science degree in Human Biology from the Philipps-University of Marburg-Germany in 1988 and a Ph.D. from the Institute for Immunology of the Ludwig-Maximilians-University of Munich Germany in 1992. From 1992 to 1997, he served his post-doctoral fellow at the Institute for Clinical Molecular Biology of the GSF-Research Center for Environment and Health, in Munich Germany. From 1997 to 1998, he was employed with Ludwig-Maximilians-University of Munich, Germany, from 1998 to 2004 he was employed at the University of Regensburg, Germany, from 2004 to 2007 he was employed with Gentium, S.p.A., and from 2008 to the present time he has been employed at the Grosshadern Medical Center-University of Munich, Germany as a Professor for Interdisciplinary Stem Cell Research in the Department of Cardiac Surgery. His primary area of expertise comprises the field of Immunology, with particular emphasis on transplantation and stem cell biology. He is a co-inventor on two Patent Cooperation Treaty applications and has authored numerous publications and grants in the field of immunology, including stem cell biology and transplantation science.

3. Under his direction and control, the following experiment was performed:

Materials and Methods: The human dermal microvascular endothelial cell line CDC/EU.-HMEC-1 (HMEC) was kindly provided by the Centers for Disease Control and Prevention

(Atlanta, GA) and has been established as previously described.[1] HMECs were cultured in MCDB131 medium supplemented with 15% fetal calf serum (FCS), 1 µg/mL hydrocortisone (Sigma, Deisenhofen, Germany), 10 ng/mL epidermal growth factor (Collaborative Biochemical Products, Bedford, MA), and antibiotics. All cell culture reagents were purchased from Gibco BRL (Karlsruhe, Germany) unless stated otherwise. 5-fluorouracil (5-FU) was obtained from Sigma (Deisenhofen, Germany). Defibrotide was obtained from Gentium SpA (Villa Guardia (CO), Italy).

Apoptosis Assay: An established method for detecting apoptosis in human endothelial cells was performed as previously described using flow cytometry) FACScan and CellQuest software (Becton Dickinson/Pharmingen, Heidelberg, Germany).[2] Endothelial and tumor cells were left untreated or were incubated in the presence of 5-FU in descending concentrations (range: 10 g/mL to 0.1 µg/mL) in the presence or absence of defibrotide or oligotide[3] for 48 hours. Cells were then washed in phosphate-buffered saline (PBS) – 10% FCS and were stained with the necrosis-detecting dye propidium iodide (PI; 0.2 µg/mL; Sigma, Deisenhofen, Germany). Apoptotic cells were identified by PI-negative staining and by a characteristic side scatter (SSC) image distinct from that of non-apoptotic cells. At least 3 experiments per cell type were performed.

Results: Results obtained from the experiment are presented below in Figure 1 and demonstrate enablement for the protective effects of an oligonucleotide of the present invention on a patient's epithelial and/or endothelial cells from immunosuppressant-induced apoptosis and/or activation. Specifically, the data clearly shows that the addition of 5-FU induces apoptosis in HMECs. However, administering either defibrotide or oligotide[4] counteracted the 5-FU-induced apoptosis, thereby achieving protection of the endothelial cells from the apoptosis induced by the immunosuppressant.

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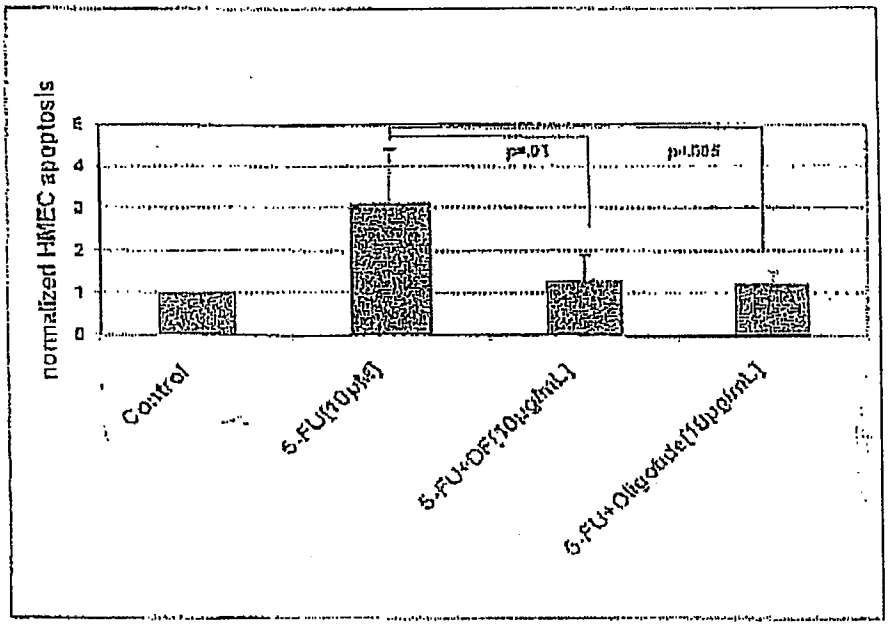
[1] Ades, E.W., Candal, F.J., Swerlick, R.A. *et al.* "HMEC-1: Establishment of an Immortalized Human Microvascular Endothelial Cell Line." *J. Invest. Dermatol.* 1992; 99:683-690.

[2] Cotter, T.G., Lennon, S.V., Glynn, J.M., Green, D.R. "Microfilament-Disrupting Agents Prevent the Formation of Apoptotic Bodies in Tumor Cells Undergoing Apoptosis." *Cancer Res.* 1992; 52:997-1005.

[3] The "oligo" as used in these experiments had the following physico-chemical and chemical characteristics: MW: 4000-10,000 Da; hyperchromicity parameter: < 10; A+T/C+G: 1.100-1.455; A+G/C+T: 0.800-1.160; specific rotation: +30±4S.

[4] *Id.* footnote 3.

Figure 1



These results demonstrate that both defibrotide or oligotide are capable of preventing 5-FU-mediated apoptosis in HMEC cells, and therefore satisfies the enablement requirement as it pertains to the terms "protective oligonucleotide" and "immunosuppressant."

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

3 Nov 2008
Date

Guenther Eissner